

APPARATUS AND METHOD FOR THE SELECTIVE ASSEMBLY OF PROTEIN

Technical Field

5 This invention relates to a method and apparatus for the selective assembly of protein.

Background Art

10 There is currently considerable interest in the development of processes and apparatus to enable the manufacture of protein filaments, fibres, ribbons or sheets. These are useful in their own right for the manufacture, for example, of sutures, threads, cords, ropes, wound or woven materials. They can also be incorporated into a matrix with or without other filler particles to produce tough and resilient composite materials. Sheets whether formed from fibres or ribbons can be stuck together to form tough laminated composites.

15 Natural silks are fine, lustrous filaments produced by the silk-worm *Bombyx mori* and other invertebrate species. They offer advantages compared with the synthetic polymers currently used for the manufacture of materials. The tensile strength and toughness of the dragline silks of certain spiders can exceed that of Kevlar, the toughest and strongest man-made fibre.

20 Spider dragline silks also possess high thermal stability. Many silks are also biodegradable and do not persist in the environment. They are recyclable and are produced by a highly efficient low pressure and low temperature process using only water as a solvent. The natural spinning process is remarkable in that an aqueous solution of protein is converted into a tough and highly insoluble material.

25 According to an article by J. Magoshi, Magoshi, m. A. Becker and S. Nakamura entitled "Biospinning (Silk Fiber Formation, Multiple Spinning Mechanisms) 11 published in Polymeric Materials Encyclopedia, by the Chemical Rubber Company, it is reported that natural silks are produced by sophisticated spinning techniques which could not, at the date of

30 writing, be duplicated by man-made spinning technologies.

Subsequently a PCT Patent Application no. WO-A-01/38614 (Vollrath & Knight) reported an apparatus and method for forming a liquid spinning solution into a solid formed product, such as a fibre. The solution is passed through at least one tubular passage having walls formed at

least partly of semipermeable and/or porous material. The semi permeable and/or porous material allows parameters, such as the concentration of hydrogen ions, water, salts and low molecular weight, of the liquid spinning solution to be altered as the spinning solution passes through the tubular passage. The inventors reported that this apparatus was suitable for the formation of fibres or sheets from all solutions of lyotropic liquid crystal polymers whether synthetic or man-made or natural or modified or co-polymer mixtures solutions of recombinant proteins or analogues derived from them or mixtures of these. These included spidroins, fibroins and recombinant protein analogues based on spidroins or fibroins.

The inventors of the PCT Application report that chemical treatment of the liquid spinning solution may occur in the tubular passage. In one example, a compartment outside of the tubular passage and separated from the tubular passage by the wall made of semipermeable and/or porous material includes 100mM Tris or PIPES buffer solution at a pH of 6.3 and 250mM potassium chloride. This last compound is described as encouraging the unfolding/refolding of the protein. However, this PCT patent application gives no indication of how this might happen nor how this can aid in the formation of filaments, fibres, ribbons or sheets.

The same authors report in an article "Liquid Crystalline spinning of spider silk", Nature, vol 410, 29 March 2001, pages 541-548, that at least one spider silk protein family encodes for polypeptides that contain a variable number of both crystalline poly-alanine domains as well as less-crystalline glycine rich domains. The authors report that although there is consensus that silk contains crystalline and non-crystalline regions, the structure of the glycine regions is imperfectly understood.

It is the inventor of the current application who first appreciated the role that glycine might have in the folding/unfolding or selective assembly of proteins.

Summary of the Invention

It is an object of the present invention to provide an improved method for the selective assembly of proteins.

These and other objects of the invention are solved by a method in which a partial protein sequence –GYG is inserted into a protein sequence. This protein sequence forms a loop structure in its native state. Subsequently, metal ions are added to thereby form the assembly. Preferably the metal ions are lithium, sodium, potassium, magnesium and/or calcium ions.

- 5 These metals are easily available and comparatively inexpensive. It has been found that the selective assembly is best achieved when the first partial sequence –GYG- is in an all-gauche conformation. The method can be used for the selective assembly of a fibrous protein, such as fibroin, spidroin and/or fibronectin. In one aspect of the inventions the first partial sequence is a member of a second partial sequence –GGYGG. In another aspect of the
10 inventions, one of the glycine molecules in the second partial sequence can be substituted with another amino acid.

The objects of the invention are also solved by an apparatus.

15 **Brief description of the drawings**

Figure 1 is a generalised schematic representation of apparatus for the formation of extruded materials from a spinning solution.

- 20 Figure 2 is a schematic cross-sectional view along the longitudinal axis of a die assembly of the apparatus shown in Figure 1.

Figure 3 is a schematic perspective view of the die assembly shown in Figure 2.

25 **Detailed Description of the Invention**

- The discovery of the way in which spiders produce dragline silk provides the basis for the invention. It has been found that by making the walls of a tubular passage at least partly permeable or porous, preferably electively permeable along the length of the tubular passage,
30 which is preferably tapered, it is possible to control properties such as the pH, water content, ionic composition and shear regime of a spinning solution in different regions of the tubular passage of a die. Ideally this enables the phase diagram of the spinning solution to be controlled allowing for pre-orientation of the fibre-forming molecules followed by a shear-

induced phase separation and allowing the formation of insoluble fibres containing well-orientated fibre-forming molecules.

Conveniently the walls defining the tubular passage are surrounded by said enclosure means to provide one or more compartments. These compartments act as jackets around the tubular passage. The tubular passage suitably has an inlet at one end to receive the spinning solution and an outlet at the other for the formed or extruded material and is typically divided into three parts arranged consecutively, the first part allowing for the pre-treatment and pre-orientation of the fibre-forming polymer molecules in the liquid feedstock prior to forming the material by draw down, the second region in which draw down of the "thread" takes place and which functions as a treatment and coating bath, and the third part has an outlet or opening of restricted cross-section which serves to prevent the loss of the contents of the "treatment bath" with the emerging fibre and to provide for the commencement of an optional air drawing stage.

It will be appreciated that any solution or solvent or other phase or phases surrounding the fibre in the second part of the or each tubular passage also serves to lubricate the fibre as it moves through and out of the tubular passage.

All or part of the length of each tubular passage typically has a convergent geometry typically with the diameter decreasing in a substantially hyperbolic fashion. According to G. Y. Chen, J.A. Cuculo and P. A. Tucker in an article entitled "Characteristic and Design Procedure of Hyperbolic Dies" in the Journal of Polymer Sciences: Part B: Polymer Physics, Vol 30, 557-561 in 1992, it is reported that the orientation of molecules in a fibre can be improved by using a die with a convergent hyperbolic geometry instead of the more usual parallel capillary or conical dies.

The geometry of substantially all or part of each tubular passage may be varied to optimise the rate of elongational flow in the spinning solution and to vary the cross-sectional shape of the formed material produced from it. The preferred substantially hyperbolic taper for part or all of the or each tubular passage maintains a slow and substantially constant elongational flow rate thus preventing unwanted disorientation of the fibre-forming molecules resulting from variation in the elongational flow rate or from premature formation of insoluble material before the spinning solution has been appropriately preoriented. A convergent taper to the

tubular passage of the die will induce elongational flow which will tend to induce a substantially axial alignment in the fibre-forming molecules, short fibres or filler particles contained in the spinning solution by exploiting the well known principle of elongational flow.

- 5 Alternatively, the principle of elongational flow through a divergent instead of convergent die can be used to induce orientation in the hoop direction that is approximately transverse to the longitudinal axis of the extruded material.

The diameter of the tubular passage may be varied to produce fibres of the desired diameter.

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The rheology of the spinning solution in the tubular passage of the die is largely independent of scale, enabling the size of the die to be scaled up or down. The convergence of the tubular passage allows a wide range of drawing rates to be used typically ranging from 0.01 to 1000 $\mu\text{m sec}^{-1}$. If fibres are being extruded they may typically have a diameter of from 0.1 to 100 μm . Typically the outlet of the tubular passage has a diameter of from 1 to 100 mm with the diameter of the inlet of the tubular passage being from 25 to 150 times greater depending on the extensional flow it is desired to produce. Tubular passages with a circular cross-section are used to produce fibres with circular cross sections. Tubular passages of alternative cross-sectional shapes can be used to produce fibres, flat ribbons or sheets of extruded materials with other, cross-sectional shapes.

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As mentioned previously, all or part or parts of the walls of the tubular passage of the die are constructed from or formed or moulded from selectively permeable and/or porous material, such as cellulose acetate-based membrane sheets. The membrane can be substituted with diethylaminoethyl or carboxyl or carboxymethyl groups to help maintain the protein containing spinning solutions in a state suitable for spinning. Other examples of permeable and/or porous material for use in the walls include hollow fibre membranes, such as hollow fibres constructed from polysulfone, polyethyleneoxide-polysulfone blends, silicone or polyacrylonitrile. The exclusion limit selected for the semipermeable membrane will depend on the size of the small molecular weight constituents of the spinning solution but is typically less than 12 kDa.

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All or part of the walls of the tubular passage can be constructed from the selectively permeable and/or porous material in a number of different ways. By way of example only, a

selectively permeable and/or porous sheet can be held in place over a groove with suitable geometry cut into a piece of material to form the tubular passage. Alternatively two sheets of selectively permeable and/or porous material can be held in place on either side of a separator to construct the tubular passage. Alternatively a single sheet can be bent round to form a tubular passage. A hollow tube of selectively permeable and/or porous material can also be used to construct all or part of the tubular passage. A variety of methods are available to shape the tube into a die as is commonly known to a craftsman skilled in the art.

The use of selectively permeable and/or porous walls of substantially all or part or parts of the tubular passage enables the proper control within desired limits of, for example, the concentration of fibre-forming material; solute composition; ionic composition; pH; dielectric properties; osmotic potential and other physico chemical properties of the spinning solution within the tubular passage by applying the well-known principles of dialysis, reverse dialysis, ultrafiltration and preevaporation.

Electro-osmosis can also be used to control the composition of the spinning solution within the tubular passage. It will be appreciated that a control mechanism receiving inputs relating to the product being formed, for example the diameter of the extruded product and/or the resistance countered in the tubular passage, such as during extrusion through the outlet of the tubular passage, can be used to control, for example, polymer concentration, solute composition, ionic composition, pH, dielectric properties, osmotic potential and/or other physicochemical properties of the spinning solution within the tubular passage.

The selective permeability and/or porosity of the walls of the tubular passage may also allow for the diffusion through the walls of further substances into the tubular passage provided that these have a molecular weight lower than the exclusion limit of the selectively permeable material from which the walls of the tubular passage are constructed. By way of example only the additional substances added to the spinning solution in this manner may include surfactants; dopants; coating agents; cross-linking agents; hardeners; and plasticisers. Larger sized aggregates can be passed through the walls of the tubular passage if it is porous rather than being simply semipermeable.

The compartments surrounding the walls of the tubular passage may act as one or more treatment zones or baths for conditioning the fibre as it passes through the tubular passage(s). Additional treatment can occur after the material has exited the outlet of the tubular passage.

5 One or more regions of the or each tubular passage may be surrounded by one or more compartments arranged consecutively so as to act as a jacket or jackets to hold solution, solvent, gas or vapour in contact with the outer surface of the selectively permeable walls of the tubular passage. Typically solution, solvent, gas or vapour is circulated through the compartment or compartments. The walls of the compartment or compartments are sealed to
10 the outer surface of the walls of the tubular passage by methods that will be understood by a person skilled in the art. The compartment or compartments serve to control the chemical and physical conditions within the tubular passage. Thus the compartments surrounding the tubular passage serve to define the correct processing conditions within the dope at any point along the tubular passage.

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In this way parameters such as the temperature; hydrostatic pressure; concentration of fibre-forming material; pH; solute; ionic composition; dielectric constant; osmolarity or other physical or chemical parameter can be controlled in different regions of the tubular passage as the spinning solution moves down the length of the die. By way of example only,
20 continuously graded or stepped changes in the processing environment can be obtained.

Conveniently a selectively permeable /porous membrane can be used to treat one side of a forming extrusion in a different way to the other side. This can be used, for example, to coat the extrusion or remove solvent from it asymmetrically in such a way that the extrusion can
25 be made to curl or twist.

All or part of the draw down process may typically occur within the die. The die can be used for forming fibres from spinning solutions containing solutions of proteins or analogues or recombinant proteins or analogues or mixtures or such proteins or protein analogues. The
30 proteins used as the spinning solution have a partial sequence or motif –GYG– incorporated into the polypeptide structure. In the native state, this forms a loop structure. In another aspect of the invention the proteins used as the spinning solution have a second partial sequence –GGYGG– in which one of the glycine molecules may be – but is not necessarily – replaced by another amino acid. When these spinning solutions are used it is necessary to

store the spinning solution at a pH value above or below the isoelectric point of the protein to prevent the premature formation of insoluble material. It will be appreciated that other constituents may be added to the spinning solution to keep the proteins or protein analogues in solution. These constituents may then be removed through the semipermeable and/or porous walls when the spinning solution has reached the appropriate portion of the tubular passage in which it is desired to induce the transition from a liquid spinning solution to solid product, e.g. thread or fibre. The spinning solution within the tubular passage is brought by dialysis against an appropriate solution containing metal ions. The solution containing metal ions could be a solution with alkali metal ions (e.g. lithium or potassium), alkaline earth metal ions (e.g. magnesium or calcium) or transition metal ions (e.g. copper, zinc or iron). These metal ion containing solutions are found to promote the formation fibres.

The draw rate and length, wall thickness, geometry and, material composition of the tubular passage may be varied along its length to provide different retention times and treatment conditions to optimise the process.

One or more regions of the walls defining the tubular passage can be made impermeable by coating their inner or outer surfaces with a suitable material to modify the internal environment in a length of the tubular passage using any coating method as will be understood by a person skilled in the art.

The inner surface of the walls of the tubular passage can be coated with suitable materials to reduce the friction between the walls of the tubular passage and the spinning solution or fibre.

The extrusion apparatus with the tubular passages surrounded by a compartment or compartments to act as jackets can be constructed by one or two stage moulding or other methods known to a person skilled in the art. It will be appreciated that a moulding process can be used to create simple or complete profiles for the tubular passage and the outlet of the die. Very small flexible lips can be formed, e.g. moulded, at the outlet to prevent the escape of the contents of the treatment bath and act as a restriction to enable an optional additional air drawing stage or wet drawing after the material has left the outlet of the die should this be required. The microscopic profile of the inner surface of the lips at the outlet can be used to modify the texture of the surface coating of the extruded material.

By way of example only, the jackets and supports for the tubular passages can be constructed from two or more components formed by injection moulding or constructed in other ways as will be understood by a person skilled in the arts. It will be appreciated that this method of construction is modular and that a number of such modules can be assembled in parallel to produce simultaneously a number of fibres or other shaped products. Sheet materials can be produced by a row or rows of such modules. Such a modular arrangement allows for the use of manifolds to supply spinning solution to the inlet of the tubular passage(s) and to supply and remove processing solvents, solutions, gases or vapours to and from the jacket or jackets surrounding the tubular passages. Additional components may be added if desired. Potential modifications to the arrangements shown will be apparent to persons skilled in the art.

Other methods of constructing extrusion apparatus in which the walls of the tubular passages are substantially or partially constructed from semipermeable and/or porous material or materials will be known by a person skilled in the art. By way of example only these include micro-machining techniques. In addition it will be appreciated that walls of the tubular passage substantially or partially constructed from semipermeable/porous material can be incorporated into other types of extrusion apparatus, such as electrospinning apparatus.

The tubular passage may be made self-starting and self-cleaning.

It will be appreciated that blockage of spinning dies during the commercial production of extruded materials is time-consuming and costly. To overcome this difficulty, the walls of the tubular passage may be constructed from an elastic material sealed into and surrounded by two or more jackets arranged in sequence. The pressure in each of these jackets can be varied independently by methods that will be understood by a person skilled in the art. Pressure changes in the jackets can be used to change the diameter of different regions of the tubular passage in a manner analogous to a peristaltic pump to pump the spinning solution to the outlet to commence the drawing of fibres or to clear a blockage. Thus a decrease in pressure in a jacket towards the outlet end of the tubular passage will dilate the elastic walls of the tubular passage within the jacket. If the pressure is now raised in a second jacket closer to the input end of the tubular passage a region of the walls of the tubular passage running through this jacket will tend to collapse forcing the spinning solution towards the outlet. Alternatively, the pressure in the spinning solution fed to the tubular passage could be increased causing the diameter of the elastic tubular passage walls to increase. It will be appreciated that both methods could be used together or consecutively. With both methods the elasticity of the

passage walls enables the diameter of the tubular passage to be increased reducing the resistance to flow. With both methods it is to be noted that increasing the pressure of the spinning solution will also assist in start up and in clearing blockages in the tubular passage. It will also be appreciated by way of example only that the use of rollers such as are used in peristaltic pumps can be used as an alternative means of applying pressure to pump dope to the outlet to commence spinning or to clear a blockage.

The pressure in the sealed compartments surrounding the tubular passage may be controlled to define and modify the geometry of the tubular passage to optimise spinning conditions.

If the tubular passage has a convergent or divergent geometry along all or part of its length, filler particles or short fibres included in the dope may be orientated as they flow through the tubular passage by exploiting the well understood principle of elongational flow. It will be understood that the substantially axial orientation of such filler particles or short fibres will be produced by a convergent tubular passage while a divergent one will produce orientation in the hoop direction that is approximately transverse to the long axis of the extruded material.

Both patterns of orientation confer additional useful properties on the fibre. It will be appreciated that a convergent or divergent geometry of all or part of the tubular passage will also serve to elongate and orientate small fluid droplets of an additional solvent or solution or other phase or phases or additional unpolymerised polymer or polymers present in the spinning solution as supplied to the tubular passage or arising by a process of phase separation within the spinning solution. The presence of elongated and well orientated narrow inclusions formed by either a convergent or divergent tubular passage can be used to confer additional useful properties to the extruded material.

It will be appreciated that the direct drawing down of a fibre or other formed product from liquid spinning solution within a region of the tubular passage greatly improves the molecular orientation in the final material avoiding the disorientation produced by die swell produced by other methods of forming the final material. It also greatly reduces the pressure required to form material compared with the extrusion of fibre from a conventional restriction die.

Figure 1 shows apparatus for the formation of extruded materials from a spinning solution (also called a dope) such as a protein polymer or polymer mixtures. The apparatus comprises

a dope reservoir 1; a pressure regulating valve or pump means 2 which maintains a constant output pressure under normal operating conditions; a connecting pipe 3; and a spinning die assembly 3 comprising at least one spinning tube or die which is further described in Figures 2 to 5. A take-up drum 5 of any known construction draws out and reels up the extruded material at a constant tension exiting from the outlet of the die assembly 3. The pressure regulating valve or pump means 2 may be any device normally producing a constant pressure commonly known to a person skilled in the art.

The arrangement shown in Figure 1 is purely exemplary and additional components may be added if desired.

Potential modifications to the arrangement shown in Figure 1 will be apparent to persons skilled in the art. In use the spinning solution is passed from the feedstock reservoir 1 at a constant low pressure by means of the regulating valve or pump means 2 via the connecting pipe 3 to the inlet of the spinning die assembly 4. The spinning solution comprises at least a protein having at least a -GYG- motif which in its native state is part of a loop structure and is in an all-gauche configuration. The -GYG- motif may be part of a -GGYGG- sequence in which one of the glycine molecules may be substituted by another amino acid. Examples of such proteins include spidroin and fibroin.

The die assembly 4 is shown in greater detail in Figures 2 and 3 and comprises a first spinning tube or die 8 upstream of a second spinning tube or die 12, the first die 8 and the second die 12 together defining a tubular passage 17 for the spinning solution through the die assembly 4. The first die 8 and the second die 12 are made of semipermeable and/or porous material, as described previously.

The die 8 is held at its upstream end by a tapered adaptor 6 positioned at the inlet end of the die assembly 4 and at its downstream end by a tapered adaptor 7 positioned internally in the die assembly 4. The die 8 is held at its upstream end by the adaptor 7 and at its downstream end by a spigot 13 at the outlet of the die assembly 4. The die 8 has a convergent, preferably hyperbolic, internal passage and the geometrical taper is preferably continued with the internal passage of the die 12. This can be achieved during construction by softening a semipermeable tube or die on a warmed suitably tapered mandrel, or by other methods as will be appreciated by a craftsman skilled in the art, before fitting the spinning tube or die into the

apparatus. The internal passages of the first die 8 and the second die 12 together provide the tubular passage 17 for the spinning solution from the inlet to the outlet of the die assembly 4.

A first jacket 9 surrounds the first die 8 and is intended to contain a fluid, e.g. a solvent, solution, gas or vapour to control the processing conditions within the first die 8. The first jacket 9 is fitted with an inlet 10 and an outlet 11 to control flow of fluid into and out of the first jacket 9. A second jacket 14 surrounds the second die 12 and is fitted with a fluid inlet 15 and a fluid outlet 16 to enable fluid, e.g. solvent, solution or gas, to be passed into and out of the second jacket 14 in contact with the semipermeable/porous walls of the second die 12.

As an alternative to the first die 8 shown having semipermeable walls a die may be constructed from material which is not semipermeable but which is preferably tapered, e.g. convergently, and may be temperature-controlled by circulating fluid at a predetermined temperature through the first jacket 9.

In operation the spinning solution is fed to the inlet of the die 8. As the spinning solution passes along the tubular passage 17 it is treated firstly as it passes through the die 8 and secondly as it passes through the die 12. The fluid passing through the jacket 9 may merely serve to heat or maintain the spinning solution at the correct temperature or provide the correct external pressure to the walls of the first die 8. In this case it is not essential for the walls of the first die to be made of semipermeable and/or material. The temperature of the dies 8 and 12 for the extrusion of the spinning solution should typically be maintained at a temperature of about 20°C but spinning may be carried out at temperatures as low as 2°C and as high as 40°C. The pressure of the fluid, liquid or gas, in the jackets 9 and 14 surrounding the walls of the tubular passage 17 is typically maintained at a pressure close to that at which the spinning solution is supplied to the die assembly 4. However, the pressure can be somewhat higher or lower depending on the geometry of the dies 8 and 12 and the strength of the generally flexible semipermeable and/or porous membrane. "Chemical" treatment of the dope occurs during "draw down" as the spinning solution passes through the second die 12 although chemical treatment may also occur as the spinning solution passes through the first die 8 if the walls of the latter are at least partly made of semipermeable material. In Figures 2 and 3, the abrupt pulling away of the spinning solution from the walls of the second die 12 at 12A indicates the internal draw down of the "fibre".

The pulling away of the "fibre" from the walls of the second die at 12A occurs at a place in the second die 12 where the force required to produce extensional flow to create a new surface just falls below the force required to flow the spinning solution through the die 12 in contact with the die walls. The position of 12A will depend on: the changing rheological
5 properties of the spinning solution; the rate and force of drawing; the surface properties of the second die 12; the surface properties of the lining of the second die 12; and the properties of the spinning solution and the aqueous phase surrounding the spinning solution.

It will be appreciated that the temperature, pH, osmotic potential, colloid osmotic potential,
10 solute composition, ionic composition, hydrostatic pressure or other physical or chemical factors of the solution, solvent, gas or vapour supplied to the jacket(s) control or regulate the conditions inside the tubular passage 17 as is commonly understood by a person skilled in the art. Chemicals in the fluid supplied to the jacket(s) are able to pass through the
15 semipermeable and/or porous walls of the tubular passage to "treat" the spinning solution passing therethrough. It is also possible for chemicals in the spinning solution to pass outwardly through the semipermeable and/or porous walls of the tubular passage 17. The fluids supplied to the spinning solution will obviously depend on the type of spinning solution used and the semipermeable and/or porous membranes used.

20 In one example, for the spinning of concentrated protein solutions, the first jacket 9 may - 19 contain 100 mM Tris or PIPES buffer solution, typically at a pH of 7.4, and 400 mM sodium chloride to help maintain the folded state of the protein. The second jacket 14 may contain 100 mM Tris or PIPES buffer solution at a lower pH, typically 6.3, and 250 mM potassium chloride to encourage the unfolding/refolding of the protein.

25 More generally either one of the first jacket 8 or the second jacket 14 could contain any solution having metal ions, preferably alkali metal ions (such as lithium, sodium and potassium), or alkaline earth metal ions (such as magnesium or calcium) or transition metals (including copper, zinc or iron).

30 High molecular weight polyethylene glycol can be added to the solution in both the first jacket 9 and the second jacket to maintain or reduce the concentration of water in the dope.

It will be realised that the spinning tube or die 12 can be hanked or coiled or arranged in other ways between the tapered collar 7 and the spigot 13. The diameter and cross-sectional shape or the exit 13 can be varied or adjusted to suit the diameter and cross sectional shape of the formed material. For a formed product having a circular cross-section, the typical diameter of the outlet is from 1 to 100 μm and the typical diameter of the inlet to the tubular passage would be from 25 to 150 times greater than the outlet diameter depending on the extent of the extensional flow. It will be appreciated that the arrangements and proportions shown in Figure 2 are purely exemplary and thus that additional components may be added if desired.

Potential modifications to the arrangements shown in Figure 2 will be apparent to persons skilled in the art.

The arrangements and proportions shown in Figure 2 are purely exemplary and thus additional components may be added if desired. Potential modifications to the arrangements shown in Figure 2 will be apparent to persons skilled in the art, including the provision of fewer or more dies 12 or jackets 14.

The permeability or porosity of the walls of the tubular passage 17 may be the same throughout the length of the latter. Alternatively, however, if the tubular passage 17 passes through more than one treatment zone the permeability/porosity of the walls of the tubular passage 17 may change from treatment zone to treatment zone by using different semipermeable or porous materials for the walls of the tubular passage 17. Thus the walls of the tubular passage 17 may comprise: semipermeable material of the same permeability throughout the length of the tubular passage 17; semipermeable material of different permeability for different portions of the tubular passage 17; porous material of the same porosity throughout the length of the tubular passage 17; porous material of different porosity for different portions of the tubular passage 17; or semipermeable material for one or more portions of the length of the tubular passage 17 and porous material for one or more other portions of the tubular passage 17. As mentioned above, some portions of the walls of the tubular passage may be non-permeable. By way of example only, suitable semipermeable materials are: cellulose derivatives, polytetrafluoroethylene, polysulfone, polyethylenoxide-polysulfone blends, and silicone polyacrylonitrile blends. By way of example only, the suitable porous materials are: polyacrylate, poly (lactide-co-glycolide), porous PTFE, porous silicon, porous polyethylene, cellulose derivatives and chitosan.